Histamine-Release

Components for the *in-vitro diagnostic* quantitative determination of histamine release in heparinized whole blood. This product must be used only in connection with the IBL Histamine ELISA (REF RE59221).

REF RE95000

Σ 96

2-8°C

EU: For research use only. U.S.: For research use only. Not for use in diagnostic procedures.

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1. **INTENDED USE**

Components for the *in-vitro diagnostic* quantitative determination of histamine release in heparinized whole blood.

This product must be used only in connection with the IBL Histamine ELISA *(REF RE59221).*

2. **SUMMARY AND EXPLANATION**

In humans, histamine (β-imidazole ethylamine) is the most important mediator and is mostly found in the initial phase of an anaphylactic reaction ("immediate type" allergy). Histamine is biosynthesized by enzymatic decarboxylation of histidine. In the organism, histamine is present in nearly all tissues, and it is mainly stored in the metachromatic granula of mast cells and the basophilic leukocytes. It is present in an inactive bound form and is only released as required.

Of clinical interest in the histamine determination is the quantification of the histamine release from basophilic leukocytes in allergies of the "immediate type" as well as of the histamine quantity which is present in various body fluids (plasma, urine, cell culture supernatants), after allergen administration. First contact of the organism with an allergen does not result in the initiation of a histamine release. First, specific IgE antibodies are produced which migrate to the mast cells and there they bind to the receptors. At the second allergen contact, a transformation of a B cell to a plasma cell is no longer required. The allergen directly moves to the IgE antibodies already bound to the mast cells and binds to these antibodies. The mast cell responds by histamine secretion from its granula.

3. **TEST PRINCIPLE**

Heparinized whole blood samples are incubated with different concentrations of the suspected allergen. Release of histamine will occur upon stimulation of basophilic granulocytes depending on their sensibility to the allergen. The released histamine in the supernatant is subsequently determined using a specific plasma immunoassay, the Histamin ELISA *(REF RE59221)* purchased in connection with this kit. This histamine value is related to the 100% control (= Total Histamine) and the blank value (= Spontaneous Release).

The determination of the in vitro release of histamine represents a sensitive and specific method as well as a suitable addition to routine diagnostic procedures including conventional skin testing and radioallergosorbent tests (RAST) for the determination of specific IgE antibodies in serum of atopic patients. In addition, this test also detects the "releasability" of the cells.

The direct detection of mediator substances like histamine during an allergic reaction is not only of scientific interest but also of practical significance in connection with a specific antagonistic therapy. This test is also very suitable for the analysis of pathophysiological responses to drugs, chemicals and other compounds which have not been evaluated for adverse side effects.

4. **WARNINGS AND PRECAUTIONS**

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.

5. **STORAGE AND STABILITY**

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
6. SPECIMEN COLLECTION AND STORAGE

Heparinized Whole Blood

24 h before drawing of blood samples the patient should avoid taking any allergy causing drugs, antihistaminics, oral corticosteroids and substances which block H₂ receptors.

The Histamine Release is performed with heparinized whole blood. The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed.

The samples should be mixed carefully immediately after collection.

Calculation of sample volumes for Histamine ELISA:
- 50 µL for total histamine content
- 200 µL for spontaneous release
- 200 µL for positive control with anti IgE
- 200 µL for each allergen concentration

Example: For a test with 2 allergens and 5 concentrations each you will need 2.45 mL of blood.

| Storage: | 18-25°C |
| Stability: | 24 h |
| keep away from heat or direct sun light. | do not cool. at 2-8°C the leucocytes would clot.

7. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Symbol</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x</td>
<td>RELEASEBUF</td>
<td>Release Buffer lyophilized Piperazinediethansulfonic acid in buffer with 0.01 % thimerosal.</td>
</tr>
<tr>
<td>1 x</td>
<td>ANTI IGE</td>
<td>Anti-IgE lyophilized Anti-human IgE. Polyclonal sheep antibody.</td>
</tr>
<tr>
<td>1 x 20 mL</td>
<td>HYPOMED</td>
<td>Hypotonic Medium Ready to use. Salt solution.</td>
</tr>
</tbody>
</table>

8. MATERIALS REQUIRED BUT NOT SUPPLIED

Additionally to the materials required for the Histamine ELISA the following materials are needed:
1. Disposable glass test tubes (12 x 75 mm)
2. Centrifuge; ≥ 700 x g
3. Water bath, 37°C
4. Ice bath

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. The relative centrifugal force (g) is not equivalent to rounds per minute (rpm) but it has to be calculated depending on the radius of the centrifuge.
10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

<table>
<thead>
<tr>
<th>Dilute / dissolve Component</th>
<th>with</th>
<th>Diluent</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>RELEASEBUF</td>
<td>20 mL</td>
<td>bidist. water</td>
<td>Let stand for 15 min.</td>
<td>2-8° C</td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mix without foaming.</td>
<td>≤ 20° C</td>
<td>1 mon</td>
</tr>
<tr>
<td>ANTI IGE</td>
<td>2 mL</td>
<td>RELEASEBUF (reconstituted)</td>
<td>Let stand for 15 min.</td>
<td>2-8° C</td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mix without foaming.</td>
<td>≤ 20° C</td>
<td>1 mon</td>
</tr>
</tbody>
</table>

10.2. Preparation of Allergens

Prepare several 10-fold dilutions (e.g. from $10^{-1}$ to $10^{-5}$) using a stock allergen solution of 1 mg/mL (e.g. stock solution in 0.9% NaCl or PBS Buffer).

$$
10^{-1} = 30 \mu L \text{ stock allergen solution (1 mg/mL)} + 270 \mu L \text{ Release Buffer}
$$

$$
10^{-2} = 30 \mu L \times 10^{-1} \text{ Dilution} + 270 \mu L \text{ Release Buffer}
$$

$$
10^{-3} = 30 \mu L \times 10^{-2} \text{ Dilution} + 270 \mu L \text{ Release Buffer}
$$

$$
10^{-4} = 30 \mu L \times 10^{-3} \text{ Dilution} + 270 \mu L \text{ Release Buffer}
$$

$$
10^{-5} = 30 \mu L \times 10^{-4} \text{ Dilution} + 270 \mu L \text{ Release Buffer}
$$

11. TEST PROCEDURE

11.1. Reaction of Whole Blood with Allergen Dilutions (in glass tubes)

1. Label glass tubes according to the following scheme for each allergen and each patient sample. Pipette Anti-IgE, Allergen Dilution, Whole Blood and Release Buffer according to the scheme into the respective tubes. Swirl whole blood samples gently before pipetting.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Anti-IgE</th>
<th>Allergen Dilution</th>
<th>Whole Blood</th>
<th>Release Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>-</td>
<td>200 µL</td>
<td>200 µL</td>
<td>-</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>-</td>
<td>200 µL</td>
<td>200 µL</td>
<td>-</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>-</td>
<td>200 µL</td>
<td>200 µL</td>
<td>-</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>-</td>
<td>200 µL</td>
<td>200 µL</td>
<td>-</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>-</td>
<td>200 µL</td>
<td>200 µL</td>
<td>-</td>
</tr>
</tbody>
</table>

Spontaneous Release

2. Mix tubes carefully, avoiding drops at the tube wall.

3. Incubate 60 min at 37°C in a water bath.

4. Stop the release by incubating for 10 min in an ice bath.

5. Centrifuge 10 min at 700 x g.

6. Withdraw 100 µL of the clear supernatant for the acylation step of the Histamine ELISA. Avoid taking cells from the pellet.

11.2. Total Histamine

In order to release the total histamine from the leucocytes.

1. Pipette 50 µL of each heparinized whole blood sample into glass tubes.

2. Pipette 950 µL of Hypotonic Medium into each tube.

3. Incubate 60 min at 37°C in a waterbath.

4. Vortex. Withdraw 100 µL for the acylation step of the Histamine ELISA.

11.3. Storage of Samples

The supernatants of the respective samples can be stored at 2 - 8 °C for one day. For longer storage up to one week freeze at -20°C. Avoid repeated thawing and freezing.
11.4. Acylation for the ELISA (in glass tubes)

Use the Plasma-Standards for whole blood.
Use the U/C-Standards for serum-free cell culture supernatants.
In order to detect any influences of the allergen preparation, one or more of the allergen dilutions should be run as additional allergen control in the assay.

The acylation is performed according to the following scheme.

<table>
<thead>
<tr>
<th>Plasma Standards (for whole blood)</th>
<th>Release Supernatant and total Histamine</th>
<th>Allergen Dilution</th>
<th>Control Plasma 1+2 (of ELISA kit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Release Supernatant and total Histamine</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Allergen Dilution</td>
<td>-</td>
<td>50 µL</td>
<td>-</td>
</tr>
<tr>
<td>Kit Controls</td>
<td>-</td>
<td>-</td>
<td>50 µL</td>
</tr>
<tr>
<td>Release Buffer</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Indicator Buffer</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Vortex.

Acylation Reagent

| 20 µL | 20 µL | 20 µL | 20 µL |

Vortex.

Assay Buffer

| 0.75 mL | 0.75 mL | 0.75 mL | 0.75 mL |

Vortex.

Incubate 30 min at RT (18-25°C).

11.5. Histamine ELISA

Perform the Histamine ELISA following the chapter TEST PROCEDURE of the Histamine ELISA instructions (REF RE59221).

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

Due to the dilution factor of 1:2 for standards, the histamine concentration of the unknown samples (spontaneous release, positive control, release supernatant, allergen control) can be read directly from the standard curve.

For the total histamine the value has to be corrected by multiplying with 10 (due to the 20-fold dilution during histamine release).

The result of the spontaneous release has to be subtracted from the unknown samples and the total histamine. Calculate the percentage of each sample, regarding the total histamine content as 100 %. Plot a curve of the percentage release against the allergen concentration on semi-logarithmic paper.

The degree of cellular sensitization (HR50) is defined as the allergen concentration which leads to a 50 % histamine release in relation to the total histamine. If the release is lower than 50 %, the HR30 (allergen concentration, which is capable to release 30 % of the total histamine) can be used.

A typical example of a histamine release experiment is given below.
**Typical Results of a Histamine Release Experiment**

![Histamine Release Graph](image)

**Conversion:**

Histamine (ng/mL) x 8.997 = nmol/L

**14. EXPECTED VALUES**

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total histamine in whole blood</td>
<td>&lt; 59 ng/mL (in EDTA whole blood after lysis with Hypotonic Medium when measuring total histamine only but not allergen-induced Release)</td>
</tr>
<tr>
<td>Spontaneous Release</td>
<td>This value should be &lt; 5 % of the total histamine content. Higher values indicate a damage of cells. Non-allergy persons have a spontaneous release of 1-2 ng/mL.</td>
</tr>
<tr>
<td>Positive Control</td>
<td>This value should be &gt; 5 % of the total histamine content.</td>
</tr>
<tr>
<td>Allergen-induced Release</td>
<td>Any positive signal greater than 5 % release (after subtraction of the spontaneous release value) has to be regarded as allergen specific positive.</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own range of normal values.

**15. LIMITATIONS OF THE PROCEDURE**

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

**16. PERFORMANCE**

See instructions for use of the Histamine ELISA (REF RE59221).

**17. PRODUCT LITERATURE REFERENCES**

1. Carlos D. Infection and Immunity (2009) Histamine plays an essential regulatory role in lung inflammation and protective immunity in the acute phase of Mycobacterium tuberculosis infection
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Cat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθµός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθµός -Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιµοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sunlight. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται µακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

**LIABILITY:** Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2011-07-01